

## The Impact of Folic Acid and B<sub>12</sub> Administration on Growth, Wool Traits and Some Hormones Concentrations of Male Arabi Lambs

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This study aimed to investigate the effects of folic acid and vitamin B<sub>12</sub> administration on the growth and wool traits in male lambs. A total of fifteen animals was used from 1<sup>st</sup> February 2015 to 30<sup>th</sup> April 2015, divided into three treatment groups. The first group served as a control, the second group received a folic acid dosage equivalent to 0.5 mg per kilogram of body weight per head, and the third group received a vitamin B<sub>12</sub> dosage equivalent to 0.1 mg per kilogram of body weight per head. The vitamins were administered orally twice a week. The results showed a significant improvement ( $P<0.05$ ) in final body weight, daily gain, and total weight gain in the vitamin B<sub>12</sub> and folic acid groups compared to the control group. Additionally, there was a significant ( $P<0.05$ ) increase in all wool characteristics, except for crimp number, in the folic acid group compared to the control group. The study demonstrated significant relationships between body weight and every aspect of wool quality except for fiber length. There was a significant ( $P<0.05$ ) increase in the concentrations of growth and thyroxine hormones in the vitamin B<sub>12</sub> and folic acid groups compared to the control group, particularly in the second and third months of the experiment. No significant difference in insulin hormone concentration among the groups was observed during the 90-day experiment. In conclusion, administering vitamin B<sub>12</sub> and folic acid are orally administered twice a week to improve growth, increase hormone concentrations (thyroxine and growth hormone), and improve wool quality.

**Keywords:** Vitamins, sheep, body weight, metabolic hormone, wool characteristics.

### INTRODUCTION

Sheep play a critical role in the agriculture industry as they provide meat, wool, milk, and other products (Al-Sayegh and Al-Kass, 2006). In general, a deficiency in providing ruminant animals with their vitamin needs, especially during stages of intensive production or pregnancy, may cause symptoms such as decreased meat and milk productivity, body growth, and hormone production (Frye *et al.*, 1991). Vitamins are essential for various physiological processes, including the metabolism of proteins and energy, improved nutrient utilization, increased appetite and feed conversion efficiency, and growth and development in mammals (Rafeeq *et al.*, 2020). The vitamin requirements in animals and humans greatly depend on their health, physiology, and productivity, such as milk, meat, wool, hair, egg production, and reproduction., most ruminants do not suffer from a deficiency of the B-vitamin group due to the ability of rumen microorganisms to synthesize these vitamins and their availability in the consumed feeds, however, under certain conditions such as stress and high production, the need for

additional vitamins has been recently established to meet their requirements, for example, vitamin B<sub>12</sub> cannot be synthesized in the rumen when cobalt is not available in the diet (McDowell, 2006). Water-soluble vitamins essential for numerous physiological processes (include folic acid and vitamin B<sub>12</sub>), functions as a cofactor in several enzymatic activities related to amino acid metabolism, DNA synthesis, and cell division. It is also essential for the creation and maintenance of genetic material, making it crucial for rapid cell growth and division (Bailey and Gregory, 1999; Wagner, 2001).

Folic acid improves metabolic health, reproductive characteristics, and pregnancy outcomes in sheep (Almahdawi and AL-Shimmery, 2019). Almahdawi (2019) concluded in their study on ewes and their lambs that the reproductive and productive performance of pregnant ewes is improved by folic acid and cobalt together, this combination increases daily milk production, promotes weight gain, improves feed conversion efficiency at weaning, and reduces anemia while increasing red blood cell synthesis. Vitamin B<sub>12</sub>, through the vitamin B<sub>12</sub>-dependent enzyme methylmalonic-

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CoA, assists in the creation of red blood cells, maintains the proper functioning of the neurological system, and improves the metabolism of carbohydrates, fatty acids, and amino acids, it also enables propionate entrance into gluconeogenesis and the Krebs cycle (McDowell, 2000). One study suggested that folic acid increases milk production in cows, whereas the dose of vitamin B<sub>12</sub> does not. However, the combination of both vitamins significantly improves feed conversion efficiency (Graulet *et al.*, 2007).

In some countries, such as Australia, New Zealand, and some Central Asian countries, wool production is considered a significant financial return, increasing the yearly income of farmers, the qualitative and physical characteristics of wool are the most important commercial factors that affect the prices of wool and consumer demand, which determine the nature and quality of manufactured textiles (Doyle *et al.*, 2021). Wool quality and traits, such as fiber length, fiber diameter, staple length, color, crimps, and fleece weight, have a considerable impact on the textile industry and its market value (Champion and Robards, 1999). Factors such as age, sex, breed, nutrition, and management control wool growth (Holman and Malau-Aduli, 2012). The weight of crude and clean wool, fiber length, staple length, and the number of crimps is considered characteristics that determine wool production in sheep (Gelaye *et al.*, 2021). Generally, nutrition and food components, including vitamins, impact wool traits (Russell, 2002). Few studies have investigated the association between folic acid and B12 vitamins in wool growth. Therefore, the objective of this study was to investigate the effects of folic acid and vitamin B<sub>12</sub> administration on growth, wool characteristics, and some metabolic hormone concentrations in male Arabi lambs.

## MATERIALS AND METHODS

This research on animals was carried out by the College of Agriculture at the University of Basrah, from 1 February 2015 to 30 April 2015 (three months). 15 male lambs, born in October 2014, between the ages of 3 and 4 months of the Arabi breed, a breed that is popular in southern Iraq, having a starting body weight of 14.56 kg were used in the study.

**Animals and experiment design:** The animals were randomized into three groups of 5 lambs each. The first treat was the control group, second group was given a dose of 0.5 mg/kg body weight/head of folic acid, and the third group received a dose of 0.1 mg/kg body weight/head of vitamin B<sub>12</sub> (Both vitamins are produced by Lovate health sciences Company Inc. / U.S.A.). Vitamins were taken orally two times per week. All the animals were healthy and free from diseases and parasites. Feeding troughs and drinking basins were provided for each group of animals in a semi-shaded pen (3x3 m<sup>2</sup>). The animals received barley at a rate of 3% of their body weight, with hay available ad libitum throughout the experiment (collective feeding). The animals' weights were

measured using a field scale (0-35 kg) before to the experiment's beginning (initial weight), and then every two weeks to adjust the amount of barley provided in the diet based on the achieved body weights. The aim of this was to compute the average daily and total weight gain.

**Collecting wool samples:** Wool samples were collected from the left shoulder, 10 cm<sup>2</sup> in from the belly and top lines before the experiment began and again at its conclusion (Taherpour *et al.*, 2012). A sensitive balance (less than three grams) was used to weigh the crude wool samples. Then, the samples were washed with clean water and dried away from air waves for five days to remove vegetable matter content, and after ensuring that it was free of impurities and dust, it was weighed and considered as the weight of the clean wool. Three random patterns were taken from each sample, placed on a white sheet of paper, and the distance between the two ends of each pattern was measured using a ruler (15 cm) to record the fibre length. Then the patterns were taped with adhesive tape to record the length of the staple. The number of crimps was determined by eye, and every up and down on the fibre was considered one crimp.

**Collecting blood samples:** Blood samples have been collected from each animal's jugular vein before the start of the experiment and every month, with a volume of 8 ml. The samples were placed in plastic tubes containing a clotting agent (gel) and were clotted for 3 hours at the temperature of the room. After that, centrifugation was used out at 5000 rpm for 15 minutes to the separate the serum, which was frozen at -20C°, until laboratory testing. Following the guidelines in the kit manual, specific measuring kits provided by Monobind Inc. - USA Company were used for measuring growth hormone, thyroxine, and insulin concentration in the serum. All hormone concentrations were estimated by enzyme-linked immunosorbent assay (ELISA) method using a device Biotek Instruments ELx800 (U.S.A.: Model No. 6880G01EA). Hormone concentrations were measured at the device using a wavelength of 450 nm.

**Statistical analysis:** The effects of various treatments on the attributes under investigation were examined using a completely randomized design (CRD), the significance of the differences between the examined means was assessed using the Least Significant Difference (LSD) test, with a significance level of (0.05). SPSS, (2019) was used for statistical analysis. The following mathematical analysis is done on the data:

$$y_{ij} = \mu + T_i + E_{ij}$$

Where:  $y_{ij}$  = value observed  $j$  in transaction  $I$ ,  $\mu$  = the overall mean of the trait,  $T_i$  = effect of treatment  $3 = i$  ( $i$  coefficients),  $E_{ij}$  = effect of experimental observational error  $j$  which is assumed to be normal, and standard distributed with a mean of zero and a variance of  $\sigma^2_e$ .

## RESULTS



**Body Growth:** The impact of folic acid and vitamin B<sub>12</sub> levels on the growth of male Arabi lambs is presented in Table 1. In the vitamin B<sub>12</sub> and folic acid treatment groups, the final body weight (23.16 kg and 22.40 kg, respectively), daily weight gain (98.00 g and 82.44 g, respectively), and overall weight gain (8.82 kg and 7.42 kg, respectively) were all significantly ( $P<0.05$ ) greater compared to the control group (19.50 kg, 56.11 g, and 5.05 kg, respectively) at the end of the experiment.

**Wool traits and their correlations:** Table 2 illustrates a significant ( $P<0.05$ ) increase in the weight of both crude and clean wool, fibre length, and crimps number in the vitamin B<sub>12</sub> group compared to the control group. Moreover, the crude wool weight showed a significant increase in the vitamin B<sub>12</sub> group compared to the folic acid group. However, after 90 days of the experiment, there was no significant variation in fibre length between the groups.

There is significant variation ( $P<0.05$ ) between the treatments, as indicated by the various letters in a row.

Table 3 shows significant relationships between body weight and all wool quality traits. Additionally, significant

correlations were observed between crude wool weight and all studied traits except fiber length, despite a negative correlation between the number of crimps and fiber length.

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**Growth Hormone:** Throughout the experiment, the concentration of growth hormone gradually increased (Fig.1). Moreover, there was a significant increase ( $P<0.05$ ) in the vitamin B<sub>12</sub> and folic acid groups compared to the control group during the second and third months of the experiment. Specifically, the concentration of growth hormone significantly increased in the groups treated with vitamins during the second (5.89 ng/ml for folic acid and 7.87 ng/ml for vitamin B<sub>12</sub>) and third (9.66 ng/ml for folic acid and 9.00 ng/ml for vitamin B<sub>12</sub>) months, compared to the control group (5.11 ng/ml and 7.07 ng/ml, respectively).

**Table 1. Effect of administering folic acid and vitamin B<sub>12</sub> on the growth of Arabi male lambs (mean  $\pm$  standard error).**

Traits	Control	Folic acid	Vitamin B <sub>12</sub>	Sig. level
Initial body weight (kg)	14.45 $\pm$ 1.76	14.98 $\pm$ 1.09	14.34 $\pm$ 1.05	N.S.
Final body weight (kg)	19.50 $\pm$ 1.8 b	22.40 $\pm$ 1.7 a	23.16 $\pm$ 2.1 a	*
Average daily gain (g)	56.11 $\pm$ 5.89 b	82.44 $\pm$ 4.56 a	98.00 $\pm$ 4.06 a	*
Average total weight gain (kg)	5.05 $\pm$ 0.90 b	7.42 $\pm$ 1.01 a	8.82 $\pm$ 1.12 a	*

There is significant variation ( $P<0.05$ ) between the treatments, as indicated by the various letters in a row.

**Table 2. Effect of administering vitamin B<sub>12</sub> and folic acid on some wool traits before and after the experiment in Arabi male lambs (mean  $\pm$  standard error).**

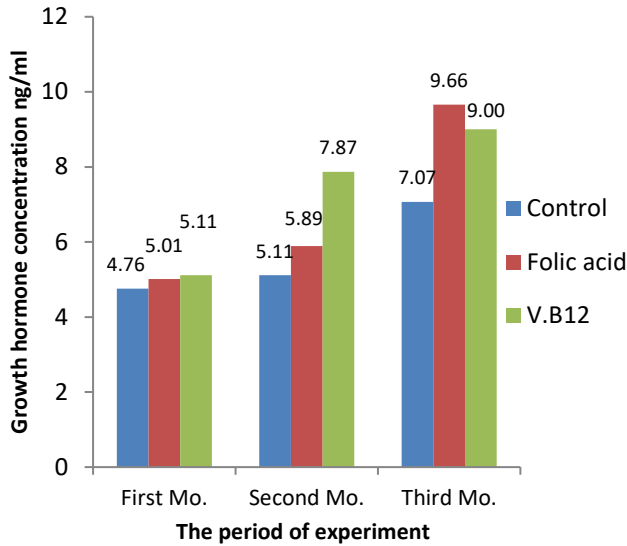
Traits		Control	Folic acid	Vitamin B <sub>12</sub>	Sig. level
Crude weight wool (g)	Before	0.142 $\pm$ .023	0.143 $\pm$ .033	0.146 $\pm$ .012	N.S.
	After	0.165 $\pm$ .010 b	0.207 $\pm$ .024 b	0.283 $\pm$ .013 a	*
Clean weight wool (g)	Before	0.122 $\pm$ .022	0.118 $\pm$ .031	0.116 $\pm$ .019	N.S.
	After	0.242 $\pm$ .011b	0.377 $\pm$ .033 a	0.449 $\pm$ .043 a	*
Fiber length (cm)	Before	2.51 $\pm$ 0.13	2.60 $\pm$ 0.12	3.13 $\pm$ 0.23	N.S.
	After	2.71 $\pm$ 0.11	3.11 $\pm$ 0.23	3.03 $\pm$ 0.31	N.S.
Staple length (cm)	Before	3.03 $\pm$ 0.33	3.87 $\pm$ 0.12	3.17 $\pm$ 0.31	N.S.
	After	3.23 $\pm$ 0.11 b	3.90 $\pm$ 0.21 a	4.11 $\pm$ 0.22 a	*
Crimps number	Before	2.33 $\pm$ .01	2.67 $\pm$ .01	2.33 $\pm$ .01	N.S.
	After	2.66 $\pm$ .01 b	2.76 $\pm$ .01 a	3.00 $\pm$ .01 a	*

**Table 3. Correlations between body weight and some qualitative wool traits.**

Traits	Final weight	Crude weight wool	Clean weight wool	Staple length	Fiber length
Crimps number	0.76**	0.49*	0.14	0.10	-0.11
Fiber length	0.36	0.30*	0.15	0.23	
Staple length	0.80**	0.14	0.60*		
Clean weight wool	0.66*	0.94**			
Crude weight wool	0.77*				

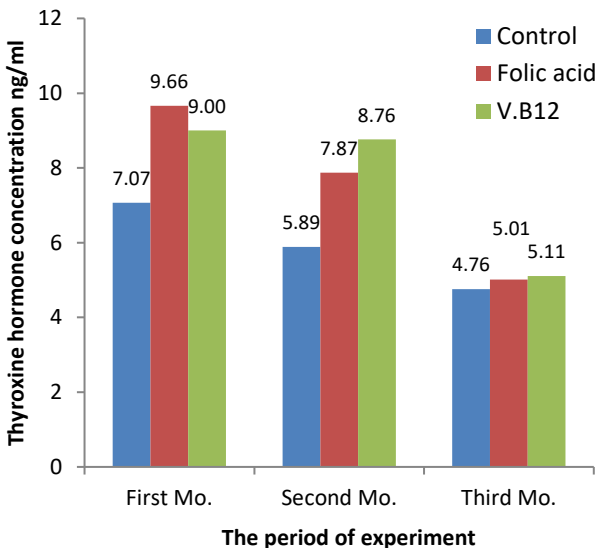
\*Significant correlation ( $P<0.05$ ). \*\*Highly significant correlation ( $P<0.01$ ).





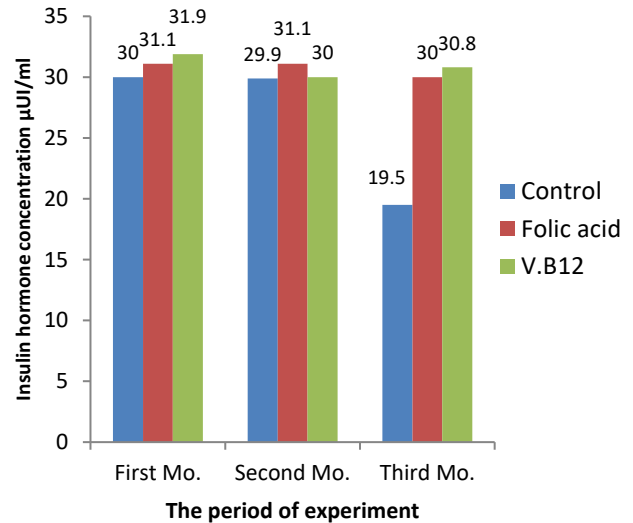
**Figure 1. Effect of administering vitamin B<sub>12</sub> and folic acid on growth hormone concentration during experiment in Arabi male lambs.**

**Thyroxine Hormone:** Thyroxine hormone concentrations gradually decreased from the first to the third month in all experimental animals (Figure 2). Additionally, there was a significant ( $P<0.05$ ) increase in thyroxine concentration in the vitamin B<sub>12</sub> group during the second month and in the folic acid group during the third month compared to the control group. However, thyroxine hormone levels did not significantly differ among the vitamin B<sub>12</sub> and folic acid groups throughout the experiment.



**Figure 2. Effect of administering vitamin B<sub>12</sub> and folic acid on thyroxine hormone level during experiment in Arabi male lambs.**

**Insulin Hormone:** Throughout the 90-day investigation, there was no significant difference observed between the groups in the concentration of insulin (Figure 3). The lowest concentration of the hormone was recorded in the control group during the third month of the experiment (29.5  $\mu$ UI/ml), while the highest concentration was recorded in the group treated with folic acid during the first (31.1  $\mu$ UI/ml) and second (31.1  $\mu$ UI/ml) months of the experiment.



**Figure 3. Effect of administering vitamin B<sub>12</sub> and folic acid on insulin hormone level during experiment in Arabi male lambs.**

## DISCUSSION

The lambs treated with vitamin B<sub>12</sub> and folic acid exhibited improved final body weight, daily weight gain and total weight gain compared to the lambs of the control group. The reason for the improved growth of the lambs that were given folic acid and vitamin B<sub>12</sub> may be attributed to the role of folic acid in tissue building of body tissues, contributing to cell division and protein synthesis, ultimately leading to increased animal growth (Kolb *et al.*, 1999). Additionally, vitamin B<sub>12</sub> helps convert propionate to succinyl-CoA, crucial for glucose release through the Krebs cycle, thereby increasing the energy sources necessary for animal growth (Taoka *et al.*, 1994). Both vitamins also play essential roles in nucleic acid formation (DNA and RNA) and protein deposition in body tissues by enhancing amino acid absorption (Almahdawi, 2018). This finding agrees with that of (Li *et al.*, 2020), who recorded an important increase in the rate of weight growth rate of lambs given folic acid as opposed to the untreated group. It also aligns with (Almahdawi, 2019) who found that lambs given folic acid twice weekly gained weight at a significantly faster than lambs in the control group, in spite of folic acid treatment improved body condition compared to the



control group and the vitamin B<sub>12</sub> group. Duplessis *et al.*, (2017) reported that intramuscular injections of folic acid and vitamin B<sub>12</sub> did not influence in the body weight of dairy cows, either independently or collectively. In addition, (Almahdawi and AL-Shimmery 2019) discovered that folic acid treatment alone had little impact on the weight of Awassi lambs, but that when combined with cobalt, it resulted in a notable improvement in final body weight at weaning age, the synergistic effects of cobalt and vitamin B<sub>12</sub> in tissue development, along with growth hormone's role in immune system activation and improved animal health, have been suggested as the cause of this, which in turn has a good impact on animals' growth. As for the results of (Graulet, *et al.*, 2007), who recorded a statistical increase in the weight of dairy cows when given vitamin B<sub>12</sub> alone or in combination with folic acid as compared to the control group and with folic acid alone.

Table 2 indicated that lambs treated with vitamin B<sub>12</sub> had improved most wool characteristics compared to the control group. The direct impact of vitamins, including folic acid and vitamin B<sub>12</sub>, primarily observed through their role in stimulating cell growth, cell division and synthesizing amino acids, as they contribute to the production of the coenzyme methyl cobalamin, which is necessary for methionine metabolism, this leads to an increase in protein metabolism in the body (Albers *et al.*, 2002) and increase the activity of wool follicles. The indirect effect is that wool is one of the products influenced by the overall body growth (Behrem and Gul, 2022). In our current study, the groups treated with these vitamins presented a significant increase in weight compared to the control group (Table 1), so it is natural to expect improvements in wool characteristics (crude and clean wool weight, fibre length and staple length. Previous studies have also shown a strong relationship between body weight and various wool properties (Kasim and Al-Oramary, 2005; Safari *et al.*, 2005; Bagkesen and Kocak, 2018). Furthermore, the increase in wool growth and improvement in its characteristics can also be attributed to a higher concentration of growth hormone (Figure 1). Folic acid and vitamin B<sub>12</sub> levels are essential for normal pituitary gland function and growth hormone release (plays a role in transmission amino acids between muscle mass and wool), ultimately affecting wool growth and it is physical traits (Smith *et al.*, 2002; Zhao and Sun, 2010, Wynn *et al.*, 1988). According to (Johnsson *et al.*, 1985), the growth rate of the wool was substantially faster in lambs given daily injections of growth hormone (0.1 mg/kg live weight) for 12 weeks,. On the other hand, the increase in the number of crimps can be attributed to the increased staple length resulting from vitamin treatment, enhancing the metabolism of cells responsible for wool growth, or to the increase in fleece weight (as observed in Table 1).

The results presented positive correlations between crude wool weight and all studied traits except fiber length, these findings are consistent with those of (Behrem and Gul, 2022),

who showed a significant relationship between body weight and greasy weight, and staple length, however, they did not find the body weight and fiber length are correlated in Merino crossbred sheep in Turkey. Similarly, to (Kasim and Al-Oramary 2005) found a highly significant correlation between fiber length and staple length in their study on the Hamadani sheep breed, also, they reported significant correlations between body weight and staple length, as well as a positive statistical correlation between fiber length and crimp number. Additionally, (Safari *et al.*, 2005) noted a fleece weight has a beneficial relationship with clean wool weight as well as a positive correlation between body weight and fiber length and staple length. However, a different study found that yearling clean wool weight and yearling fleece weight had positive correlations with body weight, while, there was a negative correlation between body weight and yearling staple length, specifically at birth weight and adult weight of Merino sheep (Mortimer *et al.*, 2017).

During the experiment, the growth hormone concentration was gradually increased (Figure 1). Growth hormone is a peptide hormone produced by the anterior pituitary gland that is responsible for promoting overall body development. It is produced and secreted by somatotrophic cell and regulates a number of physiological processes, including metabolism and growth (Younes, 2008). Additionally, growth hormone promotes protein synthesis by enhancing the absorption of amino acids and stimulates the activity of hormones responsible for maintaining glucose levels within their normal range, such as insulin (King, 2006), as a result, it is involved in growing animals. In our present study, the weight of lambs gradually increased as the experiment progressed (Table 1). (Aljubouri *et al.*, 2021) discovered a considerable rise in the concentration of growth hormone in lambs from birth to 6 months of age in two breeds (Karakul and Awassi).

There was a significant increase in the concentration of growth hormone in vitamin B<sub>12</sub> and folic acid groups when compared with the control group in the second and third months of the experiment. The increase in growth hormone levels can be attributed to vitamins' ability to increase the number of proteins available or the level of fatty acids that are volatile in the rumen, both of which promote the synthesis of peptide hormones like growth hormone (Smith *et al.*, 2002; Zhao and Sun, 2010). This result is agreed with that of (Li *et al.*, 2020), who suggested that giving folic acid caused a significant rise in the serum concentration of growth hormone in the vitamin-treated lambs compared to the control group, they concluded that the vitamin enhances lamb growth by increasing digestibility and the secretion of hormones, including growth hormone. Also, (Kilany *et al.*, 2022), suggested that pregnant (heat-stressed) cows treated with folic acid at doses of 5 and 10 g/kg twice a week had significantly increased in growth hormone levels than the control group, (especially in the fourth week of pregnancy). Whereas, the level of growth hormone in dairy cows' blood serum between





birth and four months was not affected by folic acid, according to (Petitclerc *et al.*, 1999).

Thyroxine hormone concentrations gradually decreased from the first to the third month in all experimental animals. The reason for this result can be attributed to the fact that thyroid hormones are associated with the natural growth of the body by activating biological interactions within the body, thyroid hormones are also critical for maintaining the metabolic rate, which makes them important for controlling growth (Ingole *et al.*, 2012). Water-soluble vitamins, including folic acid and vitamin B<sub>12</sub>, are essential for the body as they act as co-factors in various biological activities, and they are particularly involved in the synthesis and metabolism of thyroxine (hormone produced by the thyroid gland), these vitamins contribute to the proper functioning of the thyroid gland and the increased activity of thyroid cells, ensuring the success of metabolic processes in the body (Hurley and Doane, 1989). Aljubouri *et al.* (2021) found that male lambs of two breeds (Karakul and Awassi) had significantly lower thyroxine concentrations between birth and six months of age. Kassim and AL-Hellou, (2018) confirmed that thyroxine concentration is a higher in the growing lambs compared to the adult sheep.

There was no significant difference between the groups in the concentration of insulin in our study. Duplessis *et al.* (2017) mentioned that although insulin hormone significantly decreased in folic acid-treated cows compared to the untreated ones during the last three days of pregnancy, it gradually increased on the seventh postpartum day as compared to the control group (without folic acid), they suggested that folic acid improves the energy status of these cows.

**Conclusion:** From present results, it can be concluded that administering vitamin B<sub>12</sub> and folic acid orally twice a week significantly improved growth, certain hormone concentrations (thyroxine and growth hormone), and qualitative wool traits. Providing adequate levels of vitamin B<sub>12</sub> and folic acid in male lambs is essential to maximize growth and optimize wool traits. Understanding the mechanisms by which these vitamins affect wool growth traits is needed in the future to improve management practices in the sheep industry.

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**Ethical statement:** The authors acknowledge that the animals were treated within national animal welfare

**Availability of data and material:** We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere?

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